Chapter 1

General introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an established treatment in various hematologic malignancies, non-malignant hematologic diseases, immune disorders and metabolic disorders in both adult and pediatric patients. Allo-HSCT has the potential to replace the affected stem/progenitor cells, resulting in a properly functioning immune-hematologic system. In certain metabolic diseases, donor hematopoiesis will restore delivery of leukocyte-derived enzymes.

Pharmacotherapy is an essential part of the allo-HSCT procedure. When a patient receives an allo-HSCT, first the patient is prepared for the transplantation in the so-called conditioning phase. In this phase the patient receives immunosuppressive drugs with the goal to cause suppression of the host immune system to allow donor cell engraftment and prevent rejection of the transplant. Furthermore, the patients receives myelosuppressive drugs to create "space" in the bone marrow of the patient for donor hematopoietic stem/progenitor cells and if applicable, maximum reduction of malignant cells. Figure 1.1 gives an overview of the HSCT procedure and the risks involved.

Following conditioning, the patient reaches aplasia and the donor stem cell graft is administered. In the post-HSCT phase the goal is engraftment of donor cells and restoring the immune system and hematopoiesis. During aplasia and the early post-transplant period patients are highly susceptible to infectious complications. Therefore, patients are cared for in isolation rooms and receive antibacterial, antiviral and antifungal prophylaxis.

Next to infections, graft-versus-host disease (GvHD) is another major risk after allo-HSCT which affects 15-25% of pediatric HSCT recipients, despite routine administration of pharmacological prophylaxis.[1,2] The immune cells of the graft react with host tissue cells especially in the skin,

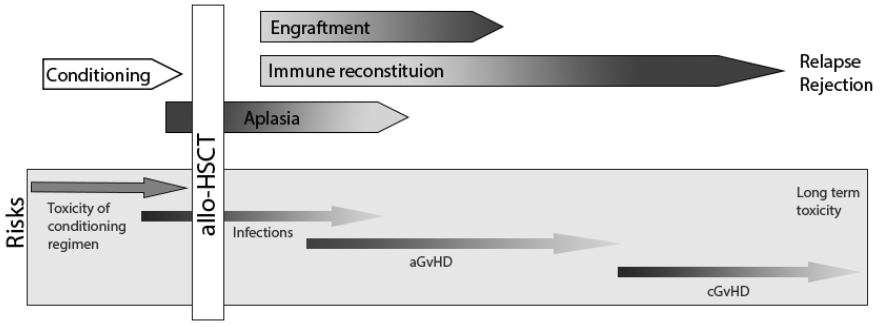

Figure 1.1 Overview of the allo-HSCT procedure and risks involved. Allo-HSCT: Allogeneic hematopoietic stem cell transplantation, aGvHD and cGvHD: acute and chronic graft-versus-host disease.

gastro-intestinal tract and liver.[3] Systemic treatment with high-dose glucocorticoids is currently the gold standard as first line treatment. Unfortunately, approximately half of the patients respond to this therapy.[4]

In recent years, improvements in different parts of the transplantation procedure have indeed made an allo-HSCT a curative treatment for an increasing number of diseases. Improvements in HLA-typing, and with that donor matching, treatment of infectious complications and optimization of conditioning regimens have improved the safety of allo-HSCT. Despite these improvements infections, conditioning regimen related toxicity and aGvHD are still major causes of severe side effects and transplant related mortality.[5]

Optimization of current drug therapies holds the potential to improve the outcome and safety of allo-HSCT. The goal of this thesis is to optimize busulfan- and treosulfan-based conditioning regimens and acute GvHD treatment with glucocorticoids by applying pharmacokinetic and pharmacogenetic profiling. Studies are performed in adults and pediatric patients. It is known that drug disposition and the pharmacodynamic effect can vary between these two populations, due to differences in body composition, metabolic capacity and maturation of enzyme function.[6] Therefore, findings in adults cannot be directly extrapolated to pediatric patients.

In the first part of this thesis the focus is on individualization of busulfan- and treosulfan-based conditioning. In **chapter 2** an overview is given of the current strategies to optimize busulfan and treosulfan therapies in pediatric allo-HSCT. Evidence has been provided that busulfan exposure is related to both HSCT efficacy and toxicity.[7,8] Therefore, therapeutic drug monitoring is applied to target busulfan exposure in the individual patient. However, a significant interpatient variability in pharmacokinetics of busulfan remains, which might be explained by pharmacogenetic variation between patients.[9–11] In **chapter 3** the role of single nucleotide polymorphisms in genes encoding for glutathione-S-transferases on busulfan clearance is investigated in adults patients undergoing an allo-HSCT. In **chapter 4**, a comprehensive pharmacogenetic analysis of busulfan PK in adult patients is performed; the DMET (drug metabolism and transport) array is applied to identify genetic markers involved in drug metabolism and transport. Based on the results in adults in chapter 4, subsequently a selection of genetic markers is analyzed in pediatric patients, this is presented in **chapter 5**. **Chapter 6** and **7** focus on treosulfan-based conditioning in pediatric patients. Treosulfan is an alkylating agent and it has a similar structure to busulfan. It is currently more often applied in allo-HSCT due to its beneficial toxicity profile in comparison with busulfan.[12] The experience with treosulfan prior to allo-HSCT is limited and only a few studies were performed to investigate treosulfan pharmacokinetics in pediatric patients. Clinical outcome of HSCT using busulfan is

associated with the exposure of the drug. Therefore, we presume that clinical outcome after HSCT applying a treosulfan-based regimen might be dependent on exposure as well. In order to study the dose-effect relation of treosulfan in HSCT, both a method of bioanalysis and a population pharmacokinetic model for treosulfan are essential. In **chapter 6** the development of a reversed phase high pressure liquid chromatography (RP-HPLC) bioanalytical method to detect treosulfan in serum is described. Furthermore, a population pharmacokinetic model and limited sampling strategy were developed. **Chapter 7** demonstrates a pilot study in which pharmacokinetic parameters of treosulfan in 21 pediatric patients were related to patient characteristics, such as age, disease, etc. Next to this, early clinical outcome is studied in relation to treosulfan exposure in this pilot study. **Chapter 8** focuses on the involvement of genetic markers in glucocorticoid responsiveness. In a retrospective cohort of pediatric patients with acute GvHD, the relation between genetic markers and glucocorticoid responsiveness is studied. In this analysis the genetic markers are investigated in both donor and recipient DNA, since both sources of immune cells are involved in the process of aGvHD. **Chapter 9** describes a case of pharmacogenetic testing in an allo-HSCT patient. The case highlights the importance of proper quality control in pharmacogenetic analysis and the challenge of pharmacogenetic testing in patients that received an allo-HSCT and have a mixed hematopoietic system. The thesis ends with concluding remarks and future perspectives in **chapter 10**.

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